Evaluation and a Predictive Model of Airborne Fungal Concentrations in School Classrooms

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Exposure to airborne fungal products may be associated with health effects ranging from non-specific irritation of the respiratory tract or mucus membranes to inflammation provoked by specific fungal antigens. While concentrations of airborne fungi are frequently measured in indoor air quality investigations, the significance of these measurements in the absence of visual mold colonization is unclear. This study was undertaken to evaluate concentrations of airborne fungal concentrations in school classrooms within a defined geographic location in British Columbia, Canada, and to build a model to clarify determinants of airborne fungal concentration. All elementary schools within one school district participated in the study. Classrooms examined varied by age, construction and presence or absence of mechanical ventilation. Airborne fungal propagules were collected inside classrooms and outdoors. Variables describing characteristics of the environment, buildings and occupants were measured and used to construct a predictive model of fungal concentration. The classrooms studied were not visibly contaminated by fungal growth. The data were evaluated using available guidelines. However, the published guidelines did not take into account significant aspects of the local environment. For example, there was a statistically significant effect of season on the fungal concentrations and on the proportional representation of fungal genera. Rooms ventilated by mechanical means had significantly lower geometric mean concentrations than naturally ventilated rooms. Environmental (temperature, outdoor fungal concentration), building (age) and ventilation variables accounted for 58% of the variation in the measured fungal concentrations. A methodology is proposed for the evaluation of airborne fungal concentration data which takes into account local environmental conditions as an aid in the evaluation of fungal bioaerosols in public buildings.

Keywords: bioaerosols; determinants of exposure; fungi; indoor air quality; schools

INTRODUCTION

Evidence is accumulating of associations between environmental source microbial products and a variety of health outcomes that are similar to sick building syndrome symptoms. Fungal exposures in schools are of particular interest because of the age and susceptibility of the occupants and the possibility of long-term health outcomes such as the development of sensitivities, asthma (Taskinen et al., 1999) or other effects attributed to mycotoxin exposures (Dales et al., 1998; Savilahti et al., 2000). Despite the possibility of adverse health effects due to exposure to fungal products, no health-based exposure limits have yet been proposed. In part this is due to the difficulty of accurately characterizing cumulative fungal spore concentrations. Fungal spores are ubiquitous in the environment and vary in concentration seasonally, geographically and by diurnal cycle (Ren et al., 1999), making the interpretation of fungal concentration data problematic and regional recommendations for remediation based on airborne fungal data even more difficult. Attempts have been made to identify fungi responsible for specific symptoms [allergic (Cruz et al., 1997), inflammatory (Rylander et al., 1992) or mycotoxic (Hodgson et al., 1998)] attributed to mold exposures. However, environmental exposures are complex interactions of microbial species and dose–response relationships have not been determined for microbial mixtures.

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In Canada, Health Canada has published guidelines to interpret airborne fungal concentrations based on studies conducted in office buildings (Nathanson, 1995). These guidelines are very conservative and represent achievable fungal concentrations in mechanically ventilated buildings with effective filtration. However, these guidelines have been widely applied to other situations which may not be appropriate, for example, naturally ventilated public buildings in areas with a different climate or population density. Therefore, the present study was undertaken to determine the distribution of concentrations of fungal aerosols that may be found in normal school classrooms (not under investigation as a sick building) in a local climatic zone. A secondary goal was to evaluate predictors of indoor fungal concentrations in these classrooms. The data obtained and predictive model developed could then be used in future studies to assess buildings or rooms in the study region that may be colonized by fungi due to water leaks, condensation or flooding. Further, the general methodology described here may be useful to other regions if appropriately modified.

MATERIALS AND METHODS

The study was conducted between January 1996 and May 1997. All 39 elementary schools within one school district in British Columbia, Canada, were enrolled. The area of the school district was 34.8 square miles with an elementary student population of 13 190. Three classrooms were chosen using a stratified random sampling scheme from each school to represent a range of building styles and the presence or absence of mechanical ventilation, carpets and classroom pets. Indoor environments were not considered to be homogeneous because the majority of schools had been subjected to continued expansion, including the construction of additional wings, annexes or temporary buildings, called ‘portables’. For example, each individual school had some classrooms which were naturally ventilated, some mechanically ventilated and some not attached to the central building. The classrooms were stratified into different air handling systems within the school and to different sampling days to maximize the variation of the samples within the sampling week. All investigations began on a Tuesday so that ventilation systems began on a Tuesday so that ventilation systems were inspected. Since no filters were operating normally. The filters on all mechanical ventilations began on a Tuesday so that ventilation systems were examined. (Praxair, Mississauga, ON). Indoor temperature and relative humidity, precipitation, wind speed, wind direction and barometric pressure data were obtained from an Environment Canada monitoring station an average distance of 10 km from the school sites. For each school, site elevation, proximity to semi-agricultural operations or major arterial roadways were obtained from detailed geographic maps and observations of the general cleanliness and proximity to vegetation were graded on a numeric scale. Fixed characteristics of the buildings (construction materials, heating and ventilation systems, etc.) were obtained from school district records. Signs of moisture, water damage or fungal growth on building materials were assessed visually.

Environmental parameters

Daily averages of outdoor temperature, relative humidity, precipitation, wind speed, wind direction and barometric pressure data were obtained from an Environment Canada monitoring station an average distance of 10 km from the school sites. For each school, site elevation, proximity to semi-agricultural operations or major arterial roadways were obtained from detailed geographic maps and observations of the general cleanliness and proximity to vegetation were graded on a numeric scale. Fixed characteristics of the buildings (construction materials, heating and ventilation systems, etc.) were obtained from school district records. Signs of moisture, water damage or fungal growth on building materials were assessed visually.

Occupancy parameters

The number and activity level of occupants (time spent sitting, moderately active or active) and the amount of time the room was occupied were monitored and recorded throughout the sample day. The presence and number of live animal pets, aquaria and house plants/organic material were also recorded.

Ventilation parameters and comfort parameters

The air exchange rate or air changes/h (a.c.h.) was assessed in unoccupied rooms with doors and windows closed using a tracer gas, sulfur hexafluoride (SF$_6$), measured using a Miran 1A portable infrared spectrophotometer (Foxboro/Wilkes, South Norwalk, CT). If the room was mechanically ventilated, flow measurements were taken using a TSI model 8360 VelociCalc Plus thermal anemometer (TSI, St Paul, MN). CO$_2$ concentrations were measured using a YES 203 (Young Environmental Systems, Richmond, BC), a non-dispersive, infrared monitor, using 5 min averaging periods. The YES monitor was calibrated at the beginning and end of each sampling period using certified clean carrier gas (compressed air, 0 p.p.m. CO$_2$) and CO$_2$ calibration gas (900 p.p.m.) (Praxair, Mississauga, ON). Indoor temperature and relative humidity were measured using two external channels of the YES 203. The temperature probe used a NTC thermistor and the RH probe used a sulfonated polystyrene wafer.
Bioaerosols

Culturable mesophilic fungi were sampled using an Andersen N-6 sampling head (Graseby Andersen, Atlanta, GA) and malt extract agar (MEA) (BBL Becton Dickinson and Co., Cockeysville, MD) in 100 × 15 mm disposable Petri dishes. Duplicate samples were taken simultaneously at a flow rate of 28.3 l/min for 5 min. Replicate samples were also collected using a restrictive xerophilic culture medium, MEA supplemented with 10% NaCl. The N-6 sampling heads were mounted on tripods extended to a height of 1 m. Indoor samples were taken at the center of the room and outdoor samples were taken near the source of fresh air for the room. The timing of the samples was standardized to be immediately after the students left for the day. For each sampling day, agar media blanks were taken into the field but not opened. The media blanks were incubated as for culture plates.

Samples and blanks were returned to the laboratory for incubation for 7 days at room temperature, exposed to a seasonally variable light/dark cycle. A second set of MEA plates was incubated at 37°C for 48 h to select for thermostolerent fungi.

Colonies were counted at the end of the incubation period with the aid of a stereoscopic microscope. Colony counts were adjusted using Andersen’s positive hole correction factors (Andersen, 1958). Fungal isolates were identified to genera level using colonial morphology characteristics (5–30× magnification) and conidial structures (400–1000× magnification) using standard procedures. Colonies that did not have conidial structures or spores were grouped together as ‘sterile mycelia’.

The limit of detection of the method was based on the presence of one countable colony on the sample medium. Thus, the limit of detection (LOD) was 7 colony forming units (c.f.u.)/m³ for the 0.1415 m³ sampled and the estimated limit of quantification (LOQ) was 210 c.f.u./m³ based on a minimum count of 30 c.f.u. (Dillon et al., 1996). For the purposes of this study, values below the LOD were assigned a nominal value of 1 colony/m³ for data analysis.

Statistical analyses

Data were analyzed using SPSS version 10.0 for Windows (SPSS, Chicago, IL). The distributions of the bioaerosol concentrations were tested for normality by the Lilliefors test and the null hypothesis of normality was rejected (P < 0.001). The data were transformed to the natural log (base e) for statistical tests requiring approximately normal distributions. The distributions of the SF₆ and CO₂ data were similarly skewed and the data transformed to the natural log.

Statistical testing of multiple comparisons for continuous data was determined by ANOVA and Scheffé’s post hoc test. Categorical and proportional data were compared using the χ² test.

95% confidence intervals of the log-normal distribution

An estimate of the arithmetic mean was calculated as the maximum likelihood estimate (MLE) of the log normal distribution and upper 95% confidence limits (UCL) were calculated on the log-normal distribution. The arithmetic mean, not the geometric mean, was used as the better predictor of exposure concentration or dose for these grab samples (Mulhausen and Damiano, 1998).

Determinants of exposure to fungal aerosols

A multiple linear regression equation was created for the outcome of interest, indoor mesophilic fungal concentration. All continuous variables were tested for a linear correlation with each other (Pearson r). Independent variables that were highly correlated with each other (r > 0.2) and which could not be entered into the regression together were examined to determine the most appropriate variable to retain in the model. Similarly, all categorical variables were tested for statistically significant relationships with other independent variables and choices made as for continuous variables. Categorical variables with more than two descriptors were coded into vectors using dummy coding.

Multiple linear regression models were constructed using a standard procedure, entering those independent variables most highly related to the outcome variable in univariate analysis and which a priori were predicted to contribute to the outcome. Independent variables were retained in the multiple regression models if they were significantly related to the outcome (P ≤ 0.05) and they contributed to an increase in the adjusted R².

RESULTS

Tables 1 and 2 report the categorical and continuous variables, respectively, with statistically significant relationships for indoor mesophilic fungal concentrations. The overall geometric mean ± geometric standard deviation indoor mesophilic fungal count was 323 ± 2.8 c.f.u./m³. The overall geometric mean outdoor mesophilic fungal count was significantly higher at 446 ± 2.1 c.f.u./m³ (P < 0.01). The coefficient of variation of the duplicate samples was 15.3% for the indoor and 13.3% for the outdoor samples. All of the mesophilic indoor MEA plates had counts above the LOD and 68% were above the LOQ. Similarly, 100% of outdoor MEA plates were above the LOD and 84.6% above the LOQ. The majority of samples were above the LOQ, and all samples were entered into the model. Sample concentrations below the LOQ had a larger variance associated with the sample estimate.
The fungal groups present in 10% or more of the samples and their seasonal variations are listed in Table 3. *Penicillium* spp. were found more often indoors than outdoors (96 versus 88%, \(P < 0.05\)) and in higher concentrations indoors (45 versus 27 c.f.u./m\(^3\), \(P < 0.001\)). *Aspergillus* spp. were isolated more often from indoor than outdoor samples (\(P < 0.05\)), but in similar concentrations. *Cladosporium* spp. were the predominant fungi isolated from outdoor samples. Fungal colonies that did not produce spores were grouped together as 'sterile mycelia'. The grouping of sterile mycelia included basidiomycetes, which were not enumerated separately from other non-sporing colonies. Non-sporing fungi were commonly found in both indoor and outdoor samples, with outdoor samples having higher geometric mean counts than indoor samples (108 versus 42 c.f.u./m\(^3\), respectively, \(P < 0.001\)).

Seasonal concentrations of indoor isolates were significantly lower in winter for the predominant fungal groups with the exception of *Aspergillus* spp. and all yeast. With the exception of the grouped yeasts, isolates of fungi from outdoors also showed significant seasonal patterns; *Aspergillus* spp. were below the limit of detection during the winter sampling seasons. The concentration of *Botrytis* spp. was dramatically higher in the autumn season both indoors and outdoors.

The geometric means of indoor and outdoor thermotolerant and xerophilic fungi are shown in Table 4. Incubating plates at 37°C allowed the recovery of *Aspergillus* spp. in 58 (50%) indoor samples versus 35 (30%) samples incubated at room temperature (\(P < 0.01\)), and 60 (52%) versus 22 (19%) outdoor samples (\(P < 0.001\)). *Aspergillus fumigatus* was recovered from 57 (49%) rooms, but at very low concentrations (3 ± 4.4 c.f.u./m\(^3\)). Unlike the mesophilic fungi, the

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### Table 1. Categorical variables with statistically significant relationships for indoor mesophilic fungal concentration

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Mesophilic fungi (mean c.f.u./m(^3))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventilation used on test day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>62</td>
<td>255.8 ± 2.96</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>No</td>
<td>54</td>
<td>420.7 ± 2.48</td>
<td></td>
</tr>
<tr>
<td>School placement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Near major arterial road</td>
<td>51</td>
<td>332.0 ± 3.03</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Residential neighborhood</td>
<td>42</td>
<td>409.1 ± 2.08</td>
<td></td>
</tr>
<tr>
<td>Trees/semi-agricultural</td>
<td>23</td>
<td>195.8 ± 3.27</td>
<td></td>
</tr>
<tr>
<td>Building age/design</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1949</td>
<td>30</td>
<td>385.8 ± 1.89</td>
<td>0.001</td>
</tr>
<tr>
<td>1950–1974</td>
<td>59</td>
<td>324.1 ± 3.10</td>
<td></td>
</tr>
<tr>
<td>1975–1995</td>
<td>10</td>
<td>96.6 ± 1.67</td>
<td></td>
</tr>
<tr>
<td>Relocatable classroom</td>
<td>17</td>
<td>469.0 ± 2.75</td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>35</td>
<td>172.3 ± 2.81</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>42</td>
<td>422.8 ± 2.97</td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>39</td>
<td>422.7 ± 2.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Snow cover</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>88.6 ± 2.26</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>No</td>
<td>111</td>
<td>341.8 ± 2.73</td>
<td></td>
</tr>
<tr>
<td>Signs of moisture in room</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>56</td>
<td>244.9 ± 3.15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Old stains</td>
<td>51</td>
<td>430.7 ± 2.34</td>
<td></td>
</tr>
<tr>
<td>Current signs</td>
<td>9</td>
<td>346.9 ± 2.32</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Geometric mean ± geometric standard deviation.

\(^b\)Group significantly different from other groups by Scheffé’s *post hoc* test.

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### Table 2. Continuous variables with statistically significant relationships to indoor mesophilic fungal concentrations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation coefficient (Pearson (r))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Building age</td>
<td>−0.248</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Supply air (m(^3)/s)</td>
<td>−0.340</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Air exchange rate (a.c.h.)</td>
<td>−0.283</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Mean indoor relative humidity (%)</td>
<td>+0.396</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean indoor temperature (°C)</td>
<td>+0.194</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mean outdoor temperature (°C)</td>
<td>+0.458</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Outdoor mesophilic fungi (ln c.f.u./m(^3))</td>
<td>+0.418</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
recovery of thermotolerant fungal groups did not vary seasonally for indoor samples. The use of a xerophilic medium allowed the recovery of aspergilli belonging to the glaucus group (11% of indoor and 6% of outdoor samples), which were not recovered from MEA medium. Only 2.6% of samples incubated for thermotolerant fungi and 15.5% for xerophilic fungi were above the LOQ, therefore, no further modeling was possible for these subsets of indoor fungi.

The arithmetic average (MLE) for indoor mesophilic fungi was 547 c.f.u./m³, with a UCL of 641 c.f.u./m³. The indoor mesophilic fungal counts were statistically different by season ($P < 0.001$) and by natural versus mechanical ventilation ($P < 0.01$). The indoor fungal concentrations were greater than outdoor concentrations for 32 (28%) rooms. When the indoor/outdoor (I/O) ratios were stratified by natural or mechanical ventilation, the naturally ventilated rooms were far more likely to have I/O > 1 (72%, $P = 0.001$).

The final multiple linear regression equation is presented in Table 5. Terms describing ventilation, outdoor temperature, outdoor fungal concentration and building age were retained in the model as statistically significant predictors of indoor fungal concentrations. Alternative measures of ventilation were tried in the model (air exchange rate and exhaust flow), but none functioned as well as supply air flow. Other variables describing occupancy were tried in the model (number of occupants, room use patterns) but none were as effective in the model as indoor CO₂. Measurements of indoor relative
humidity were strongly correlated with outdoor temperature ($r = 0.596, \ P < 0.001$), and only one of the two variables could be used in the model. Outdoor temperature was chosen as the stronger predictor.

Rooms with natural ventilation tended to have higher fungal concentrations than mechanically ventilated rooms. CO₂ concentration was a surrogate of occupancy and room use and was positively associated with the indoor fungal concentration.

Windows being open for part of the day, increased outdoor temperature and outdoor fungal concentration were all related to increased indoor fungal concentration. Schools built after 1975 were more likely to have the lowest indoor fungal concentrations compared with temporary, relocatable buildings called 'portables', which had the highest. Overall, the multiple linear regression equation explained 58% of the variation in the fungal concentration data.

In this study there was no difference in fungal concentrations between rooms with or without carpets, classroom pets, aquarium or indoor plants. About half the rooms (49%) were fully carpeted. In contrast, very few rooms had resident pets (8%) or aquarium (11%). Rooms were also judged for ‘clutter’ as a potential surrogate for surfaces that would accumulate dust. A total of 27 rooms (23%) were judged to be unusually cluttered, but there were no significant differences in fungal aerosol concentration. There was no visible fungal growth on building materials or furnishings in any of the classrooms studied.

The comfort parameters of relative humidity and temperature for the majority of rooms were within acceptable ranges. With the exception of two rooms, the relative humidity in the rooms were <60%, with 44 rooms (38%) having a mean relative humidity <40%.

### DISCUSSION

In 1996, Rao et al. (1996) published a review of the current standards and guidelines for fungi in indoor air, and in 1999 the ACGIH Bioaerosols Committee published a guidance document (Macher et al., 1999). Despite the availability of these documents, the interpretation of the significance of fungal bioaerosol concentration data relies heavily on expert opinion, as there is little consensus on what constitutes an adverse effect level. The guidance documents of many agencies suggest incorporating a dual approach which includes an indoor concentration component and a comparison with a parallel outdoor concentration.

Two Canadian agencies, Canada Housing and Mortgage Corp. (Paracel Laboratories, 1988) and National Health and Welfare Canada (Nathanson, 1993), have proposed numeric guidelines based on the analysis of thousands of research samples taken in office buildings using a RCS sampler, having taken into account the ubiquitous presence of phylloplane fungi (e.g. fungi which colonize leaf surfaces, such as Cladosporium). According to these guidelines, the persistent presence of potentially pathogenic or toxigenic fungi such as Stachybotrys or Fusarium spp. is unacceptable at any concentration and the measurement of any single species >50 c.f.u./m³ or an overall concentration >500 c.f.u./m³ warrants additional investigation. In the present study, neither of these toxigenic genera were identified in the air samples. However, 13% of rooms had fungal concentrations of a single species >50 c.f.u./m³; a majority (81%) of room samples had concentrations >150 c.f.u./m³ of a mixture of species and 35% had concentrations >500 c.f.u./m³ of fungal species not Cladosporium. However, when stratified by ventilation type, 53% of the rooms with concentrations >150 c.f.u./m³ were naturally ventilated rooms. Of these naturally ventilated rooms, the majority (66%) had I/O ratios <1 and the measured indoor fungal genera were reflective of the measured outdoor fungi.

There was a dramatic increase in specific fungal groups in the autumn sampling period, both indoors and outdoors; specifically, the indoor concentrations of Cladosporium and Botrytis spp. were an order of magnitude higher than the winter sampling period, while Penicillium spp. were on average 4-fold higher. The preferred habitat of Cladosporium spp. is leaves, and for Botrytis spp. carbohydrate-rich vegetation such as fruit or berries. The schools did not have excessive vegetation directly adjacent to the buildings, however, the study region was located in a area with many open spaces of parkland and was richly supplied with wild blackberry bushes whose berries mature in late August. The representational proportion of fungal groups was consistent over the 2 years of sampling. These data would suggest that season and fungal ecology should influence the interpretation of
fungal aerosol concentration. Neas et al. (1996) found associations between decrements in peak expiratory flow rates in asthmatic children with incremental increases in fungal spore concentrations of specific genera, including Cladosporium, and Dales et al. (2000) has reported a relationship between ambient fungal spore concentration and emergency room visits for asthma in Ottawa, Canada.

Clearly, guidelines meant for use in mechanically ventilated office spaces cannot be applied to rooms which, even when mechanically ventilated, have doors opening directly to the outside or which are used as entrances/exits regularly throughout the day. It follows that naturally ventilated rooms would be reflective of concentration and composition of outdoor fungal flora. However, Burge, in Macher et al. (1999), cautions against reliance on I/O ratios alone, because interpretation requires a high level of detail of speciation of the fungi and knowledge of concentration variability over time. In addition, Spicer and Gangloff (2000) found a high percentage of Type II errors in bioaerosol analyses (failure to reject a null hypothesis when it is false) using Spearman’s rank correlation to test the null hypothesis indoor rank = outdoor rank, particularly when there were small numbers of samples per site.

A simplified approach to evaluating mesophilic fungal concentration is suggested that can be applied equally to naturally or mechanically ventilated rooms. In a stepwise manner, evaluate data for cases where indoor concentrations are higher than outdoor concentrations. Of the rooms with I/O > 1, sort the data and segregate rooms with concentrations greater than the UCL for the region. Of those rooms with concentrations higher than the UCL, the relative ranks between indoor and outdoor fungal genera can be compared. Using these criteria for this data set, only four rooms fell into the category which would require further investigation. Interestingly, the same four rooms also had fungal concentrations >1000 c.f.u./m³, the criteria level proposed by the Occupational Health and Safety Administration (1999). However, in this data set three additional rooms had concentrations >1000 c.f.u./m³ but I/O ratios <1, with indoor fungal genera reflective of the outdoor flora, and so would not require further investigation.

This study also suggested that using media or incubation conditions designed for the selection of thermotolerant or xerophilic fungal species may be a useful adjunct to allow the identification of fungi which may otherwise be overgrown or inhibited by the growth of more prolific fungi. Higher incubation temperatures allowed the identification of A. fumigatus from about half of the room samples. The concentrations were low and although A. fumigatus is often used as an indicator organism of unacceptable fungal growth, the same organism was also isolated from concurrent outdoor samples, and in this study was regarded as part of the transient flora. Many of the study schools were located near parks and a few near semi-agricultural operations. Under these circumstances, the additional information gained by selective incubation could be used proactively, as a reminder that outdoor air may carry with it organisms that are undesirable in indoor spaces. Extremely susceptible or sensitive individuals may therefore not gain relief from outdoor exposures in rooms with freely available outdoor air.

This study and the determinants model had the following limitations. Only culturable fungi were measured. Fungal spores present in air may be non-viable or may not grow on the chosen medium. Fungal spores that grow but do not sporulate on culture medium (e.g. the sterile mycelia) prevent identification of the colony and although grouped together for this analysis, represent multiple genera. Non-viable and non-culturable spores were not quantified in this survey. Non-viable spores retain their antigenicity for susceptible hosts. Total spores may be collected as for particulate matter and counted using standard microscopic protocols. Alternatively, cell wall constituents [ergosterol (Miller and Young, 1997), extracellular polysaccharides or β(1,3) d-glucan (Chew et al., 2001)] might be used as surrogate measurements of fungal mass.

Buildings examined in this study included a range of ages, construction materials and architectural styles. All elementary schools within the district were included, and within the schools rooms were chosen to represent a range of ventilation capacities. Symptom questionnaires were not administered to occupants of the rooms and although the data were intended to represent non-problematic conditions, information regarding occupant satisfaction with the indoor air quality of the rooms was not collected. The predictive model will be tested using data from other settings to ascertain the utility of the model. With the exception of a few rooms which may benefit from further investigation, the indoor mesophilic fungal aerosol enumerated in this study was reflective of the outdoor fungal flora for this geographic region when outdoor environmental factors were taken into consideration. Regions with different climates could use a similar approach after appropriate background data were collected.

In conclusion, the contribution of outdoor fungal spores to the indoor environment of school rooms was shown to be critical for the study region. The use of these data are essential in order to evaluate the status of public facilities which do not conform to guidance values obtained from mechanically ventilated office buildings. The application of guidance criteria obtained from other regions or from other building designs could result in very costly remediation of rooms which may not require remediation, but better
methods of ventilation. Scarce resources could then be used to address the obviously abnormal fungal contamination of fewer classrooms, but in a more effective manner.

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